MPX™ Blotter

Multiplex Western Blotting Accessory

USER GUIDE

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The LI-COR® MPX (Multiplex) Blotting System was designed for screening multiple samples and multiple targets on a single Western blot. The independent channels and multi-sample features of the MPX eliminate common limitations encountered with antibody cross-reactivity during standard Western blot analysis. The MPX can be used for primary and secondary antibody screening, monoclonal antibody screening, and signal transduction pathway analysis, but all common Western procedures that generate a blot of 7.0 x 8.5 cm are adaptable to the MPX format.

The MPX conserves precious and costly antibody via low-volume channel ports (160 µL maximum) that match multi-channel pipette spacing for a simple workflow. The nesting-dovetail design allows for the linkage of multiple units while processing multiple blots.

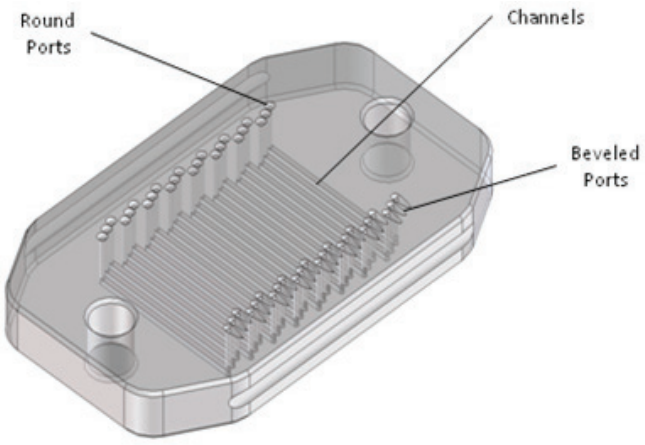
Achieving an ideal sample/target combination on the MPX begins by selecting from four gel combs designed specifically for use with the NEXT GEL (Amresco) pouring system and MPX Blotter. The collection of single-marker combs allows the user to select one, two, three, or four samples for electrophoresis and transfer to standard nitrocellulose or PVDF. Alternatively, precast gels can be used to generate blots.

Processing is as simple as clamping the blot into the MPX, which creates up to 24 independent channels. The range of usable channels per sample is relative to comb size — from 24 on a single sample to five on a four-sample blot. LI-COR's selection of IRDye® infrared dyes on any of the Odyssey® family of imagers provides a maximum of 48 targets — two per channel.

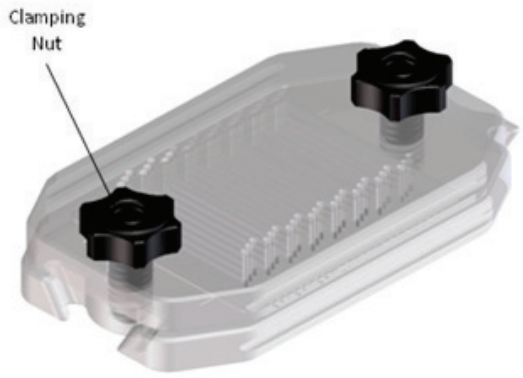
Video Instructions

Instructions marked with this icon are also available in a Flash®-based movie on the MPX Blotter CD.

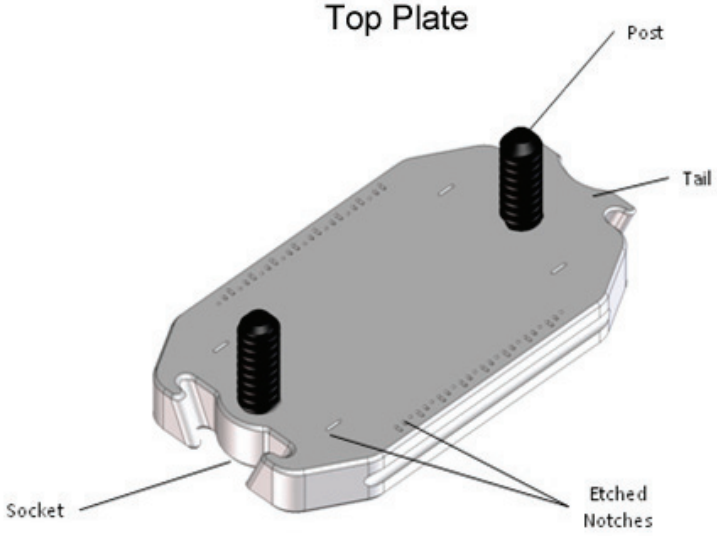
MPX Blotter



Top Plate



Assembled Unit



Base Plate

I. Suggested Materials

Workflow	Reagent / Supply	P/N
Preparing the Gel	***Use handmade poured gels or commercially-available precast gels such as NEXT GELs from Amresco.***	
	Odyssey® Blocking Buffer, Qty 3 x 500 mL	927-40003
	Odyssey Blocking Buffer, Qty 10 x 500 mL	927-40010
	Odyssey Blocking Buffer, Qty 25 x 500 mL	927-40125
	Odyssey Blocking Buffer, Qty 50 x 500 mL	927-40150
	5X NewBlot™ Nitro Stripping Buffer, 100 mL	928-40030
	5X NewBlot PVDF Stripping Buffer, 100 mL	928-40032
	NEXT GEL	
	NEXT GEL 7.5% Solution, 500 mL	M255 (Amresco)
	NEXT GEL 10% Solution, 500 mL	M256 (Amresco)
	NEXT GEL 12.5% Solution, 500 mL	M257 (Amresco)
	Combs Designed for NEXT GEL	
	Single Marker	
	1 sample well + marker well	921-00200
	2 sample well + marker well	921-00202
	3 sample well + marker well	921-00204
	4 sample well + marker well	921-00206
Preparing the Samples	4X Protein Sample Loading Buffer	928-40004
Electrophoresis	NEXT GEL Running Buffer	(Amresco)
	Odyssey Protein Molecular Weight Marker (one-color)	928-40000
	IRDye (680/800) Protein Marker (two-color)	928-40006
Blotting and Transfer	10X Tris Glycine (liquid/powder)	928-40010 or 928-40012
	Odyssey Nitrocellulose (7 x 8.5 cm sheet or 30 cm x 3 m roll)	926-31090 or 926-31092
	Odyssey Blocking Buffer and PVDF Kit	926-31098
MPX Detection	Odyssey Blocking Buffer, 500 mL	927-40000
	Casein Blocking Buffer, 500 mL	927-40200
	Primary Antibodies	
	β-Actin Mouse monoclonal antibody, 100 µL	926-42212
	α-Tubulin Mouse monoclonal antibody, 100 µL	926-42213
	β-Actin Rabbit monoclonal antibody, 100 µL	926-42210
	β-Tubulin Rabbit polyclonal antibody, 100 µL	926-42211
	IRDye Secondary Antibodies	Refer to www.licor.com/bio

Workflow	Reagent / Supply	P/N
MPX Detection (Cont.)	10X PBS (liquid/powder)	928-40018/ 928-40020
	MPX Membrane Cushion (Qty 10)	921-00120
	Western Incubation Boxes (Packs of 1, 5 & 10)	
	Small (2-78″ x 2″ x 1-3/16″)	929-97101
	5 pack	929-97105
	10 pack	929-97110
	Medium (3-1/2″ x 2-9/16″ x 1-18″)	929-97201
	5 pack	929-97205
	10 pack	929-97210
	Large (4-5/8″ x 3-1/2″ x 1-1/8″)	929-97301
	5 pack	929-97305
	10 pack	929-97310
	X-Large (6″ x 4″ x 1-1/4″)	929-97401
	5 pack	929-97405
	10 pack	929-97410
Imaging	Odyssey® CLx Infrared Imaging System	
	Odyssey Classic Infrared Imaging System	
	Odyssey Fc Chemiluminescent & IR Fluorescent Imager	
	Odyssey Sa Infrared Imaging System	

All products are available from LI-COR Biosciences, www.licor.com/bio, unless otherwise noted.

IMPORTANT: *The following methods describe suggested transfer conditions for use with the Aerius and Odyssey family of imaging systems, but are intended only as a supplement to the manufacturer's instructions. Before proceeding, familiarize yourself with the appropriate user manuals and troubleshooting guidelines. Due to the large number of factors that affect protein transfer efficiency and performance, the scope of this document is limited to the materials listed in the "Suggested Materials" section. Alternate materials may be substituted if desired; however, user optimization will be necessary.*

II. Gel Preparation

The key to a successful experiment is using the correct gel configuration for your needs.

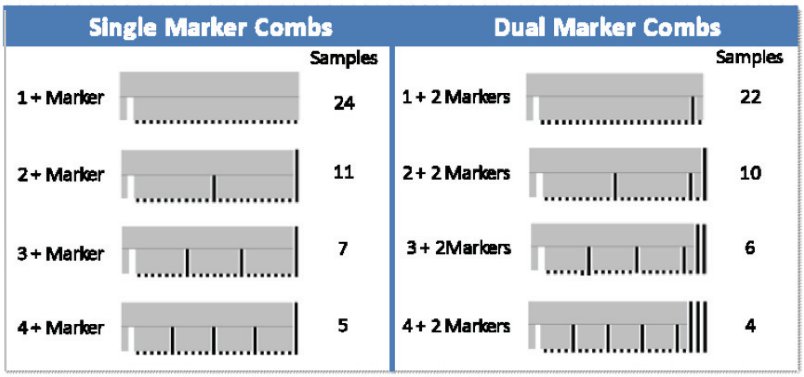
Using a Pre-Cast Gel System

Prepare the gel using the manufacturer's recommendations. Use the table as a reference for determining the number of usable ports when using the MPX with pre-cast gels. The MPX works best with single-well pre-cast gels.

Vendor	Well Designation	Sample Number	MW Marker Well	Usable Ports
Invitrogen	2D	1	Yes	19
Bio-Rad	2-D/Prep	1	Yes	21
C.B.S. Scientific	1 Well	1	No	23

Using the NEXT GEL System

Use the table as a reference for selecting the comb size based on number of samples or number of targets to be assayed.



III. Sample preparation

Dilute the sample to a final concentration of 1X in Protein Sample Loading Buffer (LI-COR® P/N 928-40004) with β-Mercaptoethanol. See package insert at http://biosupport.licor.com/docs/928-40004_4X_Protein_Loading_Buffer.pdf for more detailed instructions. Heat the sample at 95°C for 5 minutes.

IV. ELECTROPHORESIS

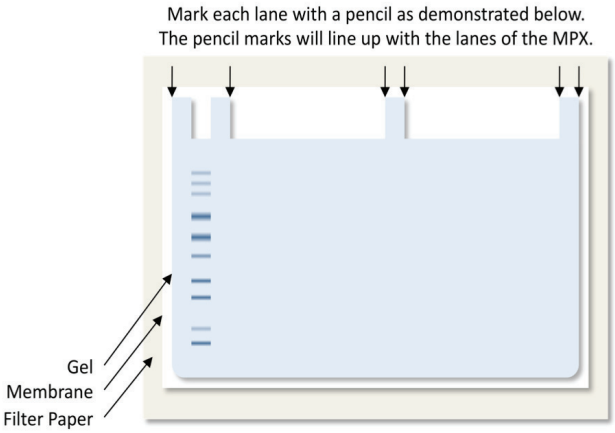
The key to a successful experiment is using the correct gel configuration for your needs.

Using the NEXT GEL System: Follow the appropriate protocol provided by Amresco.

Using Pre-cast Gel Systems: Follow the standard procedure for electrophoresis as recommended by the manufacturer.

V. TRANSFER

Always use clean forceps when handling membranes. Once electrophoresis is complete, transfer proteins to Odyssey® Nitrocellulose Membrane (LI-COR® P/N 926-31092 or 926-31090) using standard transfer procedures. Mark the outside corners of the gel and sample wells with a pencil before separating the transferred gel from the membrane. The marks help align the membrane once it is placed on the MPX. Allow the membrane to dry a minimum of one hour before proceeding with detection.


Mark each lane with a pencil as demonstrated below.
The pencil marks will line up with the lanes of the MPX.
Gel
Membrane
Filter Paper

IMPORTANT: Ink from most pens will fluoresce on the Odyssey images. Use pencil only.

VI. MEMBRANE BLOCKING

1. Place the membrane in a large (~11 x 8 cm) Western Incubation Box (LI-COR P/N 929-97301) and wet with 1X PBS for 2 minutes.

2. Decant PBS.

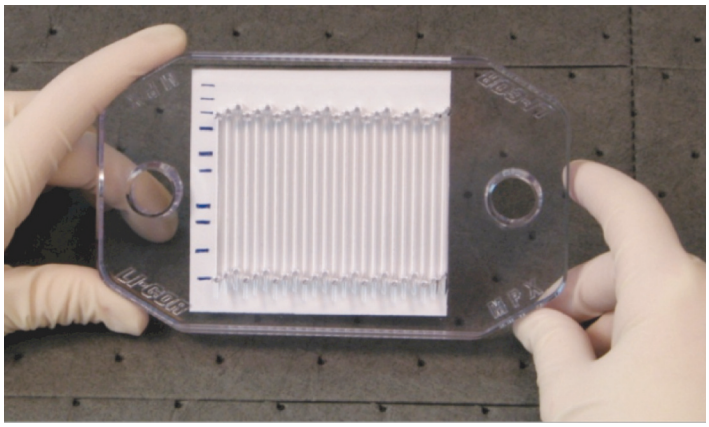
3. Cover the entire membrane with Odyssey Blocking Buffer (LI-COR P/N 927-40000) approximately 0.4 mL/cm^2, and block the membrane for 1 h at room temperature.

VII. MPX BLOTTER ASSEMBLY

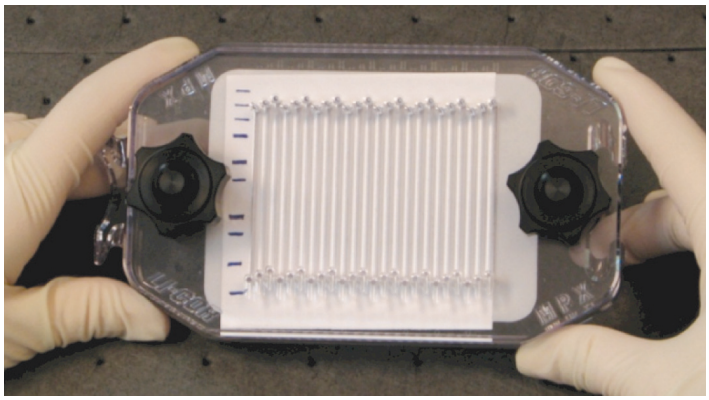
1. Place the MPX on a clean bench top. Be sure the beveled ports are closest to you and the top plate channel openings touch the base of the MPX.
2. Remove the clamping nuts and set aside.
3. Lift the top plate from the posts, flip it over, and place it face down on the bench.
   Notes:
   - The beveled port openings should be farthest from you and touch the bench.
   - The open side of the channels should face upward.
4. Place the foam cushion on the base plate. Center the posts and etched notches (right).

5. Set the base plate aside.
6. Align the wet, blocked membrane to the top plate.
   Notes:
   - The beveled ports should be farthest from you.
   - Orient the membrane so that the molecular weight ladder is on the left.
   - Hold the membrane above the top plate. Flip the membrane over and place the membrane directly onto the MPX top plate.
7. Position the membrane so the ladder lies just outside the channels. Pick up the top plate and flip it over. Check the orientation by looking through the top plate to the blot and ladder position (top right).

   IMPORTANT: Be sure the ladder is parallel to the channels, even if the membrane is not square with the top plate.



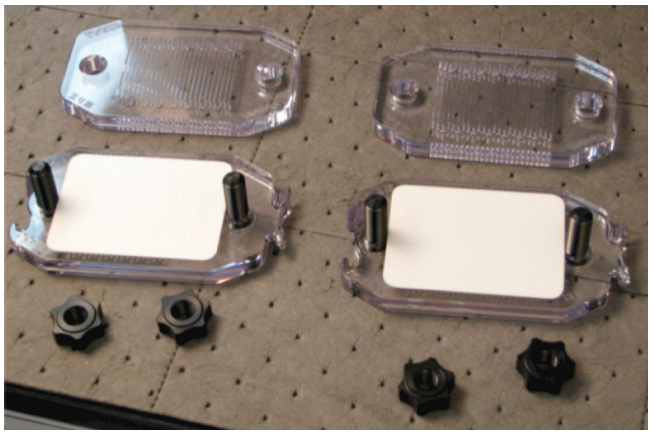
8. Slide the top plate back onto the posts, membrane side down.
   Notes:
   - Check the orientation of the membrane.
   - Be sure the foam pad lies completely underneath the membrane.
9. Place the clamping nuts onto the posts and finger-tighten simultaneously (right).



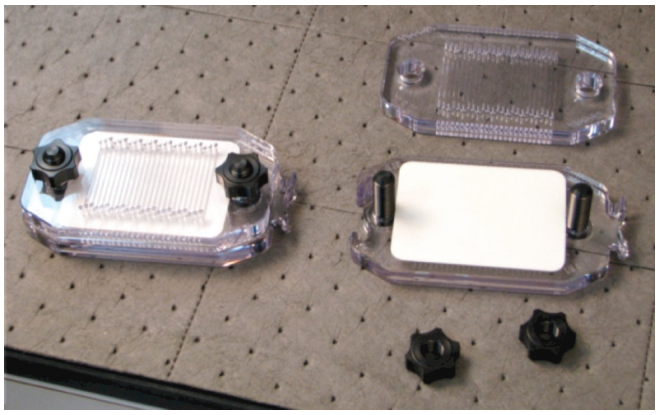
Using Multiple MPX Blotters

When using multiple MPX Blotters, it is possible to connect them by sliding the nesting–dovetail joints together.

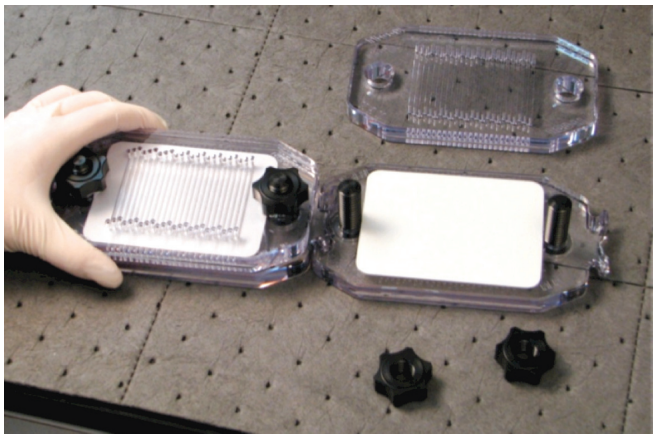
1. After placing the membranes on the top plates but before securing the tops, orient the base plates with the socket on the left and the tail on the right.



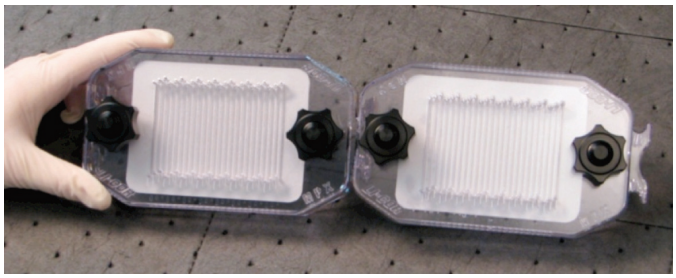
2. Assemble the left-most MPX.



3. Then slide the tail end of the assembled MPX into the socket on the base of the unassembled MPX.



4. Assemble the right-most MPX.



VIII. PRIMARY ANTIBODY APPLICATION

1. Prepare primary antibody dilutions in Odyssey® Blocking Buffer and 0.1 to 0.2% Tween® 20 as recommended by the antibody manufacturer. 200 µL of dilution will be needed for each port to which the antibody is to be applied. Dilutions should be made in a microcentrifuge tube if using a single pipette or a 96-well plate if using a multi-channel pipette. The numbers in the template represent the MPX channel to which the antibody will be applied.

	1	2	3
A	22	23	24
B	19	20	21
C	16	17	18
D	13	14	15
E	10	11	12
F	7	8	9
G	4	5	6
H	1	2	3

2. Place the MPX at an angle for easy loading and bubble clearance by propping the base with paper towels or holding the base with the non-working hand.

3. Add primary antibody using either a single or multi-channel pipette.

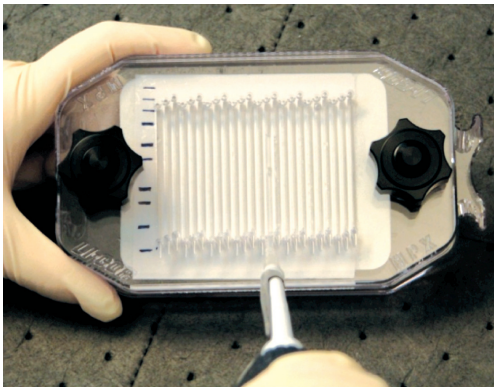
   IMPORTANT: For best results, fill all channels with liquid. Blank channels should be filled with blocking buffer or PBS to prevent drying of the well during processing.

Using a Single Pipette

1. Adjust a standard 200 µL pipette to approximately 200 µL.

2. Use a clean pipette tip and fill the pipette with primary antibody solution.

3. Place the pipette tip into the appropriate beveled port and dispense antibody solution slowly (right).



4. Watch the liquid fill the channel. If bubbles appear in the channel, stop pipetting and gently aspirate the air bubble without removing the tip. Allow the bubble to float to the top of the pipette tip, then resume pipetting.

5. The channel is full when liquid appears outside the round port hole opposite the pipette. Stop pipetting immediately and remove the tip from the port before releasing pressure on the plunger.

6. Blot excess liquid away from port openings with an absorbent cloth to avoid cross-contamination.

7. Change tips and repeat steps 1-6 until all MPX channels are full.

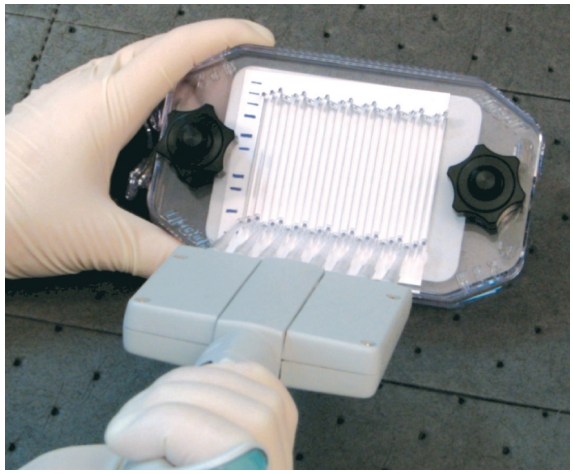
Using a Multi-Channel Pipette

IMPORTANT: The novice user may not want to use the multi-channel pipette method without having first used the single pipette method.

1. Adjust a standard multi-channel pipette (8 or 12 channel) to approximately 200 µL.

2. Position clean tips on eight consecutive tip holders, then fill pipette.

3. Place the tips into the appropriate staggered port holes and dispense primary antibody solution slowly (right).



4. Watch the liquid while channels fill. If bubbles appear in the channels, stop pipetting and gently aspirate the air bubbles without removing the tips. Allow bubbles to float to the top of the pipette tips, then resume pipetting.

5. The channels are full when liquid appears outside the round port holes opposite the pipette tips. Stop pipetting immediately and remove the tips before releasing pressure on the plunger.

6. Blot excess liquid away from port openings with an absorbent cloth to avoid cross-contamination.

7. Change tips and repeat steps 1-6 until all MPX channels are full.

IX. PRIMARY ANTIBODY INCUBATION AND WASH STEPS

1. Incubate the membrane with the primary antibody for a minimum of 1-2 hours at room temperature or overnight at 4°C with gentle shaking.

2. Remove the primary antibody solution with an 8- or 12-well multi-channel pipette, a single pipette, or vacuum aspiration.

3. Wash the MPX channels by pipetting 160 µL of PBS-T (0.1% Tween® 20) into each channel and incubate at room temperature for 5 minutes.

4. Remove the wash solution with a multi-channel pipette, single pipette, or vacuum aspiration.

5. Repeat steps 2-4 two times.

6. To prevent cross-contamination, aspirate the final wash carefully and completely before beginning the secondary antibody application. Hold the MPX at an angle to allow any excess liquid to flow to the bottom of the channel, then aspirate again.

X. SECONDARY ANTIBODY APPLICATION

Using One Secondary Antibody

1. Open the MPX; remove the membrane with forceps and wash in PBS-T for 10 minutes at room temperature with shaking.

2. Prepare secondary antibody dilutions in Odyssey® Blocking Buffer and 0.01% to 0.02% Tween 20 as recommended.

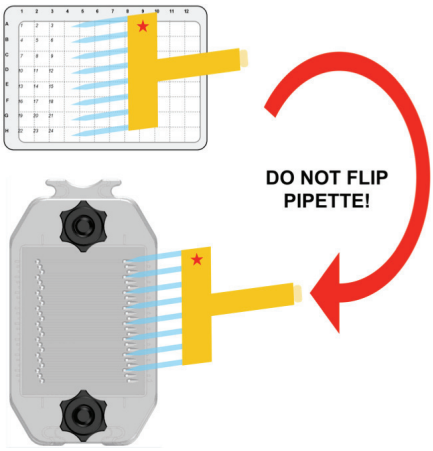
   IMPORTANT: Start with a 1:5000 dilution for secondary antibodies.

   Approximately 0.04 mL/cm^2 of diluted antibody will be needed.

3. Decant the wash solution and cover with diluted secondary.

Using Multiple Secondary Antibodies

1. Prepare secondary dilutions in Odyssey Blocking Buffer and 0.01% to 0.02% Tween 20 as recommended.
   Notes:
   - Start with a 1:10,000 dilution for LI-COR® IRDye® 800CW secondary antibodies and IRDye 680RD secondary antibodies.
   - Start with a 1:20,000 dilution for IRDye 680LT secondary antibodies.
   - 200 µL of diluted antibody will be needed for each port to which the antibody is to be applied.
   - All antibody dilutions should be premade in a 96-well plate ready for beginning.



2. Adjust a standard 8-well multichannel pipette or single channel pipette to approximately 200 µL. Use clean tips on eight consecutive tip holders, then fill the pipette.

3. Place the tip in the first port or first set of ports, then depress the plunger and watch as the liquid fills the channels.
   Notes:
   - If bubbles appear in the channel, stop pipetting and aspirate the air bubbles without removing the tips.
   - Allow bubbles to float to the top of the pipette tips, then resume pipetting.

4. Stop pipetting when liquid appears outside the round port holes opposite the pipette tips. Remove the tips before releasing pressure on the plunger.

   IMPORTANT: For best results, fill all channels. Blank channels should be filled with blocking buffer or PBS to prevent drying of the wells during processing.

XI. SECONDARY ANTIBODY INCUBATION AND WASH STEPS

1. Incubate for 30 minutes to 2 hours at room temperature with gentle shaking. Cover MPX during incubation and wash steps to protect from light. Incubation time will need to be optimized to reduce background.
2. Remove the secondary antibody solution with a multi-channel pipette, single pipette, or vacuum aspiration.
3. Wash the MPX channels once by pipetting 160 µL of PBS-T (0.1% Tween® 20) into all channels and incubate at room temperature for 5 minutes with shaking.
4. Remove the wash solution with an 8-well multi-channel pipette, single pipette, or vacuum aspiration.
5. Open MPX, remove membrane with forceps, and place into a clean wash box.
6. Add enough 1X PBS-T (0.1% Tween 20) wash solution to cover the membrane and incubate 5 minutes with shaking.
7. Repeat 2-3 more times.
8. Rinse blot before imaging for 5 minutes in 1X PBS.

XII. IMAGING

Please refer to your manual for specific information on your imager model. If using the Odyssey® CLx or Classic Infrared Imaging System, it may be beneficial in some cases to image the blot in a small pool of PBS.

XIII. MAINTENANCE AND CARE

Clean the MPX Blotter with a mild detergent and air dry after each use. A 75% ethanol solution can be used as a final rinse if necessary. If the MPX becomes contaminated with IRDye® that does not wash off with detergent, clean with a 100% solution of methanol at room temperature.

CAUTION: The MPX should NEVER be rinsed with acetone or chloroform.

CAUTION: Avoid autoclaving and heating at temperatures >50°C, as this may warp the MPX.

XIV. SPECIFICATIONS

Unit Material	Polycarbonate
Unit Dimensions	4 x 6 x 2 cm (W x L x H)
Shipping Weight	454 g (1 lb.)
Channels	24
Channel Dimensions	1.5 mm x 6 cm x 1.5 mm (W x L x H)
Channel Volume	160 µL
Membrane Size	7 x 8.5 cm
Cushion Material	Foam